UNIVERSITI PUTRA MALAYSIA

PERFORMANCE OF THREE GENERA OF ENTOMOPATHOGENIC FUNGI AS POTENTIAL MICROBIAL CONTROL AGENTS AGAINST THE FLEA BEETLE PHYLLOTRETA STRIOLATA F. (COLEOPTERA : CHRYSOMELIDAE)

TRI PUJI PRIYATNO

FP 2001 23

PERFORMANCE OF THREE GENERA OF ENTOMOPATHOGENIC FUNGI AS POTENTIAL MICROBIAL CONTROL AGENTS AGAINST THE FLEA BEETLE *PHYLLOTRETA STRIOLATA* F. (COLEOPTERA : CHRYSOMELIDAE)

By

TRI PUJI PRIYATNO

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Agricultural Science in the Faculty of Agriculture Universiti Putra Malaysia

August 2001

DEDICATION

To my wife, Yaya Suciati and my daugther, Izdihar Afaf

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Agricultural Science

PERFORMANCE OF THREE GENERA OF ENTOMOPATHOGENIC FUNGI AS POTENTIAL MICROBIAL CONTROL AGENTS AGAINST THE FLEA BEETLE *PHYLLOTRETA STRIOLATA* F. (COLEOPTERA : CHRYSOMELIDAE)

By

TRI PUJI PRIYATNO

August 2001

Chairman : Assoc. Prof. Dr. Yusof Ibrahim

Faculty : Agriculture

The striped flea beetle (FB), *Phyllotreta striolata* F. (Coleoptera : Chrysomelidae), is not only a serious pest of canola and mustard but also feed on a wide range of other brassicas. Entomopathogenic fungi (EF) are promising agent for biological control of FB and are gaining increasing attention worldwide as mycoinsecticide. The potential of three genera of EF, *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus*, has been studied in the laboratory and the field against the striped FB, *Phyllotreta striolata* F.

Surveys for FB naturally infected with EF indicated that *M. anisopliae* v. *manus* and *B. bassiana* were the potential EF in the populations of FB sampled from vegetable area at UPM's Research Park, Serdang. However, the incidence of infection was very low. Therefore, introduction of virulent isolates into a temporary habitat must be done.

Test for pathogenicity of 16 isolates of EF against adult FB found only one isolate of *M. anisopliae* (MPs) causing mortality in excess of 50%. Four isolates were tested for pathogenicity against the eggs and larvae of the FB. Two isolates of *M. anisopliae* (MPs and Cy3), one *B. bassiana* (Wls) and one *P. fumosoroseus* (Pf) were found to be highly pathogenic against the FB larvae while both isolates of *M. anisopliae* were infective against the FB eggs.

The resistance of FB adults against EF was caused by the existence of fungistatic compounds on the integument. Five straight chain fatty acids (C4, C6, C7, C8, and C9) suspected as fungistatic compounds based on analysis using Gas Chromatography were proven to inhibit conidial germination.

Two media, rice flour and sponge-rice flour medium, examined for conidial mass-production of *M. anisopliae* v. *majus* and *P. fumosoroseus* indicated that the sponge-rice flour medium was shown to be potentially efficient for mass-production of fungal spores.

Three formulations of microbial control agent (MCA), namely liquid, dust and granule, were prepared for this study using oil and glycerine, kaolin, and peat soil as carriers, respectively. The oil, glycerine, and kaolin-formulated conidia were equally significant causing higher infection on adult beetles compared to that of the control. Granules consisted of peat-formulated mycelia showed good sporulation on peat and thus have high potential as soil inoculum. However, its effectiveness was dependent on insect mobility to make contact with the conidia on peat since the peat-formulated mycelia is not an infective agent.

The conidial viability in MCA formulation was observed during storage at room temperature and under refrigeration. Propagules viability in all the formulations of MCA was very dependent on storage condition. Room termperature was detrimental to conidial and mycelial viability. In the refrigerator (4°C), conidia in glycerine and kaolin formulation still showed good viability up to 32 weeks after storage. The viability and conidiation of mycelia in granular formulation were also good when kept under refrigeration up to 32 weeks.

The most virulent *M. anisopliae* (MPs) isolate did not provide adequate protection against the FB on Chinese mustard. However, peat-formulated mycelia as soil inoculum sporulated well and survived for a long time. In the current study, it would be highly probable that *M. anisopliae* could establish well if the plots were to be continuously inoculated with the peat-formulated mycelia, thus affording an additional suppressing agent in an integrated pest management programme.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan ijazah Master Sains Pertanian

PENAMPILAN TIGA GINERA KULAT PATOGEN SERANGGA SEBAGAI EJEN KAWALAN MIKROBAL YANG POTENSIAL UNTUK MENGAWAL KUMBANG LENTING *PHYLLOTRETA STRIOLATA* F. (COLEOPTERA : CHRYSOMELIDAE)

Oleh

TRI PUJI PRIYATNO

Ogos 2001

Pengerusi : Profesor Madya Dr. Yusof Ibrahim

Fakulti : Pertanian

Kumbang lenting berjalur (KL), *Phyllotreta striolata* F. (Coleoptera : Chrysomelidae), merupakan perosak yang penting bukan sahaja pada tanaman canola dan mustard tetapi juga pada lain-lain tanaman jenis brassicas. Kulat entomopatogen (KE) merupakan satu agen kawalan biologi kepada KL dan semakin mendapat perhatian di seluruh dunia sebagai "mycoinseticide". Potensi tiga genera KE yaitu *Metarhizium anisopliae*, *Beauveria bassiana* dan *Paecilomyces fumosoroseus*, telah diselidiki keberkesanannya di makmal dan di lapang terhadap KL, *Phyllotreta striolata* F.

Hasil survei ke atas KL yang dijangkiti secara asli oleh KE mendapati *M. anisopliae* v. *manus* dan *B. bassiana* adalah KE yang berpotensi terhadap populasi KL yang telah disampel dari kawasan tanaman sayur di Pusat Taman Penyelidikan UPM, Serdang. Walau bagaimanapun kejadian jangkitan adalah sangat rendah. Oleh yang demikian pengenalan pencilan yang virulen pada habitat sementara perlu dilakukan.

Ujian kepatogenan 16 pencilan KE terhadap KL dewasa mendapati hanya satu pencilan *M. anisopliae* (MPs) yang menyebabkan kematian melebihi 50%. Ujian kepatogenan empat pencilan telah dijalankan terhadap telur dan larva KL. Didapati dua isolat *M. anisopliae* (MPs dan Cy3), satu *B. bassiana* (Wls) dan satu *P. fumosoroseus* (Pf) menunjukkan kepatogenan yang tinggi terhadap larva, manakala kedua-dua pencilan *M. anisopliae* berupaya menjangkiti telur KL.

Kekebalan KL dewasa terhadap KE adalah disebabkan oleh kewujudan sebatian fungistatik pada integumen. Lima asid lemak berantai lurus (C4, C6, C7, C8 & C9) yang disyaki sebagai sebatian fungistatik berdasarkan analisis kromatografi gas telah terbukti merencatkan percambahan konidium *M. anisopliae*, *B. bassiana* dan *P. fumosoroseus*.

Ujian penentuan penghasilan konidium *M. anisopliae* v. *majus* dan *P. fumosoroseus* ke atas dua jenis media yaitu media tepung beras dan media sponge-tepung beras menunjukkan media span-tepung beras berpotensi sebagai penghasil konidium yang cekap.

Tiga formulasi agen kawalan mikrobial (AKM), yaitu cecair, debu dan granul, telah sediakan masing-masing menggunakan minyak dan gliserin, kaolin, dan tanah gambut sebagai pembawa. Formulasi konidium dalam minyak, gliserin, dan kaolin didapati sama-sama meningkatkan jangkitan kulat ke atas KL dewasa berbanding kawalan. Granul yang terdiri dari miselium yang dibalut tanah gambut menunjukkan pembentukan konidium yang baik dan oleh itu mempunyai potensi tinggi sebagai inokulum tanah. Walau bagaimanapun, keberkesanannya bergantung kepada mobiliti serangga untuk bersentuhan dengan konidium pada tanah gambut karena formulasi miselium dalam tanah gambut bukanlah agen jangkitan.

Pemerhatian viabiliti conidium di dalam formulasi AKM telah dilakukan semasa penyimpanan pada suhu bilik dan di dalam peti sejuk. Viabiliti propagul pada semua formulasi AKM didapati bergantung kepada keadaan dalam simpanan. Suhu bilik didapati boleh merosakan viabiliti konidium dan mycelium. Di dalam peti sejuk (4°C), konidium di dalam formulasi gliserin dan kaolin masih menunjukkan viabiliti yang baik sehingga minggu ke 32 tempoh simpanan. Viabiliti dan konidiasi miselium dalam formulasi tanah gambut juga baik apabila disimpan di dalam peti sejuk selama 32 minggu.

Formulasi *M. anisopliae* (MPs) yang paling virulen tidak memberi perlindungan ke atas Chinese mustard terhadap KL di lapangan. Walau bagaimanapun, formulasi miselium dalam tanah gambut berupaya bersporulan dengan baik dan mandiri untuk jangka masa yang lama. Kajian masa kini menunjukkan *M. anisopliae* berkemungkinan tinggi untuk berkembang dengan berkesan jika plot diinokulasikan secara berterusan dengan granul, oleh yang demikian bertindak sebagai penambahan agen kawalan di dalam sesuatu program pengurusan perosak bersepadu.

ACKNOWLEDGEMENTS

Above all, I would like to thank Allah S.W.T., Most Gracious, Most Merciful, for his Compassion and Mercy.

I would like to express my deep sense of gratitude and appreciation to my supervisor, Associate Professor Dr. Yusof Bin Ibrahim, for his advice, guidance, and constructive criticisms in connection with the research and preparation and revisions of this manuscript. I also like to thank my other committee members ; Associate Professor Dr. Ahmad Said Sajap and Associate Professor Dr. Dzolkhifli Omar, thank you for your helpful insight and suggestions in this study.

I gratefully acknowledge Agriculture Research Management Project (ARMP-II) in Department of Agriculture in Indonesia for providing me the financial support which enables me to complete this degree.

A special note of thanks goes to my friends, colleagues, and staff members of Department of Plant Protection, Faculty of Agriculture, UPM.

I would like to express my deepest thanks and appreciation to my father, Miso and mother, Minah, for their encouragement, support and endless prayers during my study in Malaysia. This endeavour would not have been feasible without the sacrifice, patience, understanding and encouragement of my dearest wife Yaya Suciati and my daughter Izdihar Afaf.

I certify that an Examination Committee met on 20th August 2001 to conduct the final examination of Tri Puji Priyatno on his Master of Agricultural Science entitled "Performance of Three Genera of Entomopathogenic Fungi as Potential Microbial Control Agents Against the Flea Beetle *Phyllotreta Striolata* F. (Coleoptera : Chrysomelidae)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the examination committee are as follows :

Rohani Ibrahim, Ph.D.,
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairperson)

Yusof Ibrahim, Ph.D.,
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Ahmad Said Sajap, Ph.D.,
Associate Professor
Faculty of Forestry
Universiti Putra Malaysia
(Member)

Dzolkhifli Omar, Ph.D.,
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

MOHD. GHAZALI MOHAYIDIN, Ph.D.,
Professor/Deputy Dean of Graduate School,
Universiti Putra Malaysia

Date : 30 AUG 2001

The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Agricultural Science.

AINI IDERIS, Ph.D.,
Professor
Dean of Graduate School,
Universiti Putra Malaysia

Date :

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

TRI PUJI PRIYATNO

Date : 29 AUGUST 2001

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1

INTRODUCTION

The flea beetle belonging to the family Chrysomelidae (Coleoptera) has been reported as serious pest of canola and mustard. The two abundant species, *Phyllotreta cruciferae* (Goez) and *P. striolata* F., have been reported as the most common and prevalent insect pests distributed around the world (Varma, 1961; Elsawaf *et al*., 1965; Bonnemaison, 1965; Wylie, 1979; Burgess, 1982; Lamb and Turnock, 1982; Elliot, 1992). The most severe damage usually occurs when adult beetles emerge from the soil. They feed voraciously not only the leaves but also the seed pods of canola, mustard, and several other species of cruciferous plants. Seedlings are especially susceptible to flea beetle attack, and extensive flea beetle feeding on seedlings may destroy a crop (Lamb, 1984). They can kill plant directly by severing the hypocotyl or by eating the newly emerged meristem, or they may decrease leaf area by inflicting small, round, shot-hole wounds on cotyledons, leaves and stems (Soroka and Pritchard, 1987)). Their larvae may significantly reduce yield by feeding on the roots (Wylie, 1979). The economic impact of flea beetles on crop production varies with population densities. Yield losses of about 10% are common where flea beetles are abundant even when the crops are protected with insecticides (Bracken and Bucher, 1986).

Limited control of this pest is achieved by using cultural practices and various biological control measures (Wylie, 1984; Hazzard and Ferro, 1991; Burgess, 1982). Majority of the growers preferred applying chemical insecticides by spray, seed treatment, or in-furrow granules treatment against insects pests at seedling stage (Elliot, 1992). However, the use of chemical insecticides on crucifers presents a number of problems that may assume greater importance in the future, such as the development of resistance and environmental contamination that can dangerously affect non-target organisms (Lamb, 1989). As such, dependency on the use of chemical insecticides must be curtailed and some suitable safe alternative control methods must be found.

The adoption of integrated pest management (IPM) systems has placed biological control in a much more important role. Predators, parasitoids, and diseases have become important factors in regulating insect population. To date the effect of biological control agents on flea beetles seems to be limited (Wylie *et al.*, 1981). Lacewing larvae (*Chrysoperla carnae*), big-eyed bugs (*Geocoris bullatus*), the two-lined collops (*Collops vittatus*), the western damsel bug (*Nabis alternatus*) and the northern field cricket(*Gryllus pennsylvanicus*) are a few of the insects known to prey on flea beetles (Gerber and Osgood, 1975). The braconid wasp, *Microctonus vittatae*, parasitizes flea beetle adults; however, its overall effect on flea beetle populations is unknown (Wylie *et al*, 1981) . Unfortunately, flea beetles emerge in large number during relatively short period of time and tend to overwhelm the parasites and predators.

Biological control of flea beetles using fungal pathogens has not much been investigated. Also there are no records, of natural infections of flea beetles by fungal pathogens. Recently, however, Butt *et al.*, (1994) identified isolates of *Metarhizium anisopliae* which were highly pathogenic for the cabbage stem flea beetle (*Psylloides chrysocephala*), however, its infectivity was low against the closely related chrysomelid

Phaedon cochleariae (mustard beetle). Miranpuri and Khachatourians (1994) reported the effectiveness of *Beauveria bassiana* against flea beetle. They reported the LT_{50} values for *B. bassiana* ranged from a low of 1.9 to a high of 16.6 days, and 50-90% of the cadavers showed fungal mummification within seven days, depending upon the isolates tested.

Metarhizium anisopliae and *B. bassiana* are virulent pathogens of very wide range of soil-inhabiting Coleoptera such as *Phyllotreta* larvae that feed on the roots of plants, shrubs, and trees, because the soil ecosystems can provide favourable conditions for fungal survival, i.e. protection from solar radiation. So, they have successfully persisted in the hosts environment (Carruther and Haynes, 1986). Fungi have some advantages that make them unique among the entomopathogens. Rather than killing their host by toxigenic action following oral ingestion, they usually invade their host directly through the integument via the germ tube of a germinating spore (Steinhaus, 1963; Tanada and Kaya, 1993). The infection is not only limited to chewing insects, but also occurs in Homoptera and other arthropods with piercing-sucking mouth-parts and all stages of development of insect. In addition, they are able to persist in some soils for long periods and infect soil-inhabiting coleopteran larvae of all stages given them a distinct advantage over most chemical pesticides that do not persist in soil and frequently contaminate the environment. Therefore fungal pathogens have potentials to be developed as a suitable and safe alternative control agents in IPM programme of flea beetles.

The Plant Protection Department of UPM has a collection of isolates of *Metarhizium anisopliae* var. *majus*, *Beauveria bassiana*, and *Paecilomyces fumosoroseus* which are currently being tested on cabbage caterpillars. They are promising agent for biological control and are gaining increasing attention worldwide as mycoinsecticide. Thus, it is of prime importance to examine their usefulness as microbial control agents against the flea beetle (*P. striolata)*. The aim of this study are to survey for flea beetles naturally infected with entomopathogenic fungi and investigate the effect of oil, kaolin, glycerine, and peat soil as carrier in formulation of mycoinsecticide on viability and virulence of those fungi. The study also aimed at elucidating the efficacy of these entomopathogenic fungi when applied against the flea beetles in the field.

CHAPTER 2

LITERATURE REVIEW

2.1. Bionomic of Flea Beetle

Flea beetles feed on plants belonging to the mustard family (Cruciferae) grown throughout the world (Varma, 1961; Bonnemaison, 1964; Lamb, 1980). Eight species of flea beetles are known to attack canola, mustard, and rape seed (Jones and Jones, 1977). Of these, only the crucifer flea beetle (*P. cruciferae* Goeze) and the striped flea beetle (*P. striolata* F) are significant pests (Burgess, 1977; Kinoshita *et al*., 1979).

The economic impact of flea beetles on crop production varies with population densities (Bracken and Bucher, 1986). Yield losses of about 10 percent are common where flea beetles are abundant even when the crop is protected with insecticides. Annual crop losses in North America from flea beetles probably exceed US$300 million.

Flea beetles feed on the cotyledons, leaves, apical bud tissue, petioles, stems, roots and seed pods of crucifers (Kinoshita *et al*., 1979; Lamb, 1984). The effect of the feeding activity upon crop development varies with the part of the plant fed on, crop development, growing conditions and the intensity of the attack (Lamb, 1984). Adult beetles feed on the surface of leaves, stems and seed pods and produce small pits (Kinoshita *et al*., 1979). The tissue underneath the injury eventually withers and dies. On leaves and cotyledons, the damaged tissues break up and fall out producing a shot hole appearance (Wesdal and Romanow, 1972; Burges, 1977). Heavy infestations may severely damage cotyledons, the first leaves, petioles, and stems (Putman, 1977; Lamb and Turnock, 1982; Bracken and Bucher, 1986). The crop can usually compensate for the destruction of the individual plants, provided large portions of the crop are not totally destroyed (Bracken and bUcher, 1986). Feeding damage is most severe when beetles attack the growing point (meristem) because it limits the ability of the plant to compensate (Putman, 1977; Lamb, 1984).

Light to moderate infestations delay plant development and cause uneven maturity (Lamb, 1984). Delayed maturity may expose the crop to adverse temperatures during flowering or before the plants have matured. Uneven maturity at harvest reduces seed quality or yield. Delaying harvest to allow immature pods to ripen contributes to yield loss when over-ripe seed pods shatter during harvest. Harvesting too early produces a crop with many immature seeds containing high chlorophyll levels, affecting seed quality and yield. Most of this damage can be prevented if canola is protected from flea beetle injury during two to three weeks following emergence.

Flea beetle may also compound crop damage indirectly, by virtue of their ability to transmit diseases. *Phyllotreta* sp. have been reported to transmit turnip yellow and turnip mosaic viruses (Finch and Thompson, 1992), thus reducing the plant stands and affecting the appearance and market ability of the cruciferus crops (Kinoshita *et al.*, 1979). The corn flea beetle (*Chaetocnema pulicaria*) transmits *Erwinia stewartii*, the causal pathogen of stewart's wilt disease in maize (Munkvold *et al.*, 1996), and transmission of broom mosaic virus in barley is by *Phyllotreta vittula*. Additionally,